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Phytochemical analysis and antimicrobial investigation on leaf and fruit extracts of *L. aegyptiaca* Mill

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ABSTRACT

The study aimed to explore the phytochemical constituents and antimicrobial properties of *L. aegyptiaca* Mill. [Syn.: *Luffa cylindrica* M.Roem.] (Cucurbitaceae) leaf and fruit extracts. Standard procedures were used to measure the phytochemical constituents, and the extracts were tested against six bacterial and three fungal strains using the disc diffusion method for bacteria and the agar-well diffusion method for fungi. The results of the phytochemical analysis showed that the floral extract had higher levels of alkaloids, tannins, saponins, and oxalate. In terms of antimicrobial activity, the methanol extract of the leaf was found to be more effective than the fruit extract against the bacterial strains tested. The study suggests that the methanol extract of *L. aegyptiaca*'s leaf and fruit has significant potential for developing effective treatments for fungal diseases and more efficient antibacterial agents.

Keywords: *L. aegyptiaca*, GC-MS, FT-IR, antimicrobial activity, methanol extract.

1. INTRODUCTION

Medicinal plants represent a rich reservoir for a diverse array of medications, as noted by (Santos et al., 1995). Crude extracts derived from these plants serve not only in treating ailments in both humans and animals but also in maintaining overall health, as highlighted by (Pravin et al., 2012; Alagbe 2023; Alagbe et al., 2023). Among these plants, those belonging to the Cucurbitaceae family stand out as economically valuable resources for healthcare. Cucurbit vegetables, found predominantly in tropical and subtropical regions, serve not only as sources of food and fiber but also as indigenous medicines, as documented by (Nayar and More, 1998). One such plant of interest is Luffa aegyptiaca Mill., commonly known as sponge gourd or loofa, recognized for its therapeutic properties.

Tannin-Spitz et al., (2007) have extensively documented its antiviral, antitumor, antioxidant, anti-inflammatory, and immunomodulatory attributes. Despite its widespread use, reports on the toxicity, adverse effects, or drug interactions associated with L. aegyptiaca remain scarce. Moreover, there is limited information

available regarding the chemical composition of L. aegyptiaca seeds, despite its prevalence throughout India. Traditional medicinal practices have utilized the fruit of this plant to address various conditions such as fever, tumors, bronchitis, leprosy, serving purposes ranging from laxative to depurative, expectorant, and diuretic, as reported by.

2. MATERIALS AND METHODS

Collection and Preparation of Samples

The plant materials utilized in this study were gathered from the farm of St. Joseph's College, located in the Tiruchirappalli area (10°49'42.2"N 78°41'25.2"E), and subsequently authenticated. Both seeds and leaves were dried in the shade, powdered, sieved, and then prepared for analysis. Specifically, the dried seeds were processed into a coarse powder form and utilized for various investigations (Suryanti et al., 2015; Lucy and Abidemi, 2012; Sinha and Sharma, 2015; Madhu, 2012).

Preparation of Extracts

The powdered leaf and fruit of Luffa aegyptiaca were subjected to soaking in four different solvents for 24 hours each, repeated twice, following which they underwent filtration. The resulting filtrate was then allowed to air dry. The crude extracts obtained from the leaf and fruit samples were stored separately. The selected solvents were concentrated through an evaporation process. Phytochemical screening was carried out utilizing standard methodologies (Sofawora, 1993; Trease and Evans, 1978; Harbone, 1973).

Phytochemistry

GC-MS Analysis

Gas chromatography interfaced with a mass spectrometer (GC-MS) instrument was used to carry out GC-MS analysis. The sample was diluted 1/10 using Hexane (60-80), and 10 microlitres of the diluted sample were injected using an automatic injector (Agilent). The fuel gas used was hydrogen, and the carrier gas used was nitrogen with a flow rate of 2.50 ml/min. The column used was HP-5 (5% Phenyl Methyl Siloxane) with dimensions of 30 x 320 x 0.25. To maintain a temperature of 280°C in the injection part, the FID detector was used. The oven temperature was programmed to increase from 100°C to 200°C at a rate of 2°C/min and then from 200°C to 250°C at a rate of 3°C/min, and then maintained at 250°C till the end. The split ratio used while injecting was 60:1, with a split flow of 60 ml/min.

FT-IR Analysis

The powdered materials of selected plants were analyzed using FT-IR at the Archbishop Casimir Instrumentation Centre (ACIC) of St. Joseph's College, Trichy. The Potassium Bromide (KBr) technique and procedure was used. Each plant material was ground to a fine powder and mixed with completely dried KBr (ratio of 1/100). The mixture was then subjected to a pressure of 5x106 pa in an evacuated to produce a KBr pellet for FT-IR spectrometric analysis. The FT-IR spectrum of each sample was recorded with Perkin Elmer FT-IR Spectrum RX1.

The pellets of the sampled plants were scanned at room temperature (25±2 ºC) at a spectral range of 4000-400 cm-1. A spectral resolution of 4.0 cm-1 was set for noise reduction. The number of scans was adjusted to 15 times to obtain optimal results. The Spectrum version 5.0.2 software was used to record the spectrum of each sample. Background spectra collected under identical conditions were subtracted from the sample's spectrum. Standard FT-IR tables were used for assigning corresponding functional groups by interpreting the peaks obtained in the spectrum (Ragavendran et al., 2011).

Antibacterial and Antifungal

The extracts were tested against six different types of bacteria found in the intestines (Enterobacter aerogenes, Escherichia coli, Klebsiella pnemoniae, Proteus mirabilis, Proteus vulgaris, Serratia marcescens) and three types of fungi (Aspergillus niger, Penicillium vermiculatum, and Mucor indicus). The bacteria were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh, while the fungi were obtained from the Tropical Institute of Ecological Sciences (TIES) in Kerala. The test microorganisms were maintained on nutrient agar (for the bacteria) and potato dextrose agar (for the fungi) slopes, and kept in a refrigerator at 4°C as described in (Adesina et al., 2014).

Zone of Inhibition (mm)

The maximum antibacterial activity was observed in the ethanol and methanol extracts. Meanwhile, the methanol extracts showed the highest antifungal activity. The antifungal efficiency was tested at three different concentrations (50, 100, and 150mg/ml) using the filter paper disc diffusion method. The inhibition zone was calculated, and the minimum dimension of the no-fungal growth zone around the filter paper disc was determined (Kottai-Muthu et al., 2009).

3. RESULTS

The methanol extracts of both the leaf and fruit have been analyzed through GC-MS, resulting in the identification of over twenty-five phytochemical compounds in each extract (Figure 1 & 2). The leaf extract contains compounds such as Phenol, 2,4-Bis(1,1-Dimethylethyl)-Guanosine, β-d-Glucopyranose, 1,6-Anhydro-Bicyclo [2.2.1], Heptane-2,3-Diol, 1,7,7-Trimethyl-, (Endo, Endo)-Dodecanoic Acid, Alpha.-D-Glucopyranoside, Methyl Tetradecane, 1-Chloro-Docosane, Tetradecanoic Acid, 3-Heptadecanol, Isopropyl Myristate, Neophytadiene, Hexadecanal, and Methyl Stearate (Table 1).

On the other hand, compounds such as 5-Hydroxymethylfurfural, Anisole, P-Chloro-Phenol, 2,4-Bis(1,1-Dimethylethyl)-Eicosane, γ.-Hydroxyisoeugenol, Myristic Acid, Isopropyl Myristate, Neophytadiene, Hexahydrofarnesyl, Acetone, and Pentadecanoic Acid were identified in the fruit extract (Table 2). These compounds have various uses such as antioxidants, hypocholesterolemic, nematicide, pesticides, lubricants, antiandrogenic, and hemolytic.

Table 1 GCMS - *L. aegyptiaca* - Methanolic Extract of Leaf

S. No.	Name	Molecular Formula	Molecular Weight	Retention Time
1	5-Hydroxymethylfurfural	C6H6O3	126.111	6.073
2	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	C14H22O	206.329	9.763
3	Guanosine	C10H13N5O5	283.244	9.984
4	Beta.- D-Glucopyranose, 1,6-anhydro-	C6H10O5	162.141	10.150
5	Bicyclo [2.2.1] Heptane-2,3-Diol, 1,7,7-Trimethyl-, Endo, Endo)-	C10H18O2	170.252	10.208
6	Dodecanoic Acid	C12H24O2	200.322	10.368
7	Alpha.- D-Glucopyranoside, Methyl	C7H14O6	194.183	11.183
8	1,6-Anhydro- .Beta.-D-Glucofuranose	C6H10O5	162.141	11.531
9	1-Chlorotetradecane	C14H29Cl	232.836	11.789
10	Docosane	C22H46	310.61	12.042
11	Tetradecanoic Acid	C14H28O2	228.376	12.709
12	Loliolide	C11H16O3	196.246	12.975
13	3-Heptadecanol	C17H36O	256.474	13.262
14	Isopropyl Myristate	C17H34O2	270.457	13.465
15	Neophytadiene	C20H38	278.524	13.659
16	3,7,11,15-Tetramethyl-2-hexadecene	C20H40	280.54	13.722
17	Phytol, acetate	C22H42O2	338.576	13.952
18	Hexadecanal	C16H32O	240.431	14.192
19	7,9-Di-Tert-Butyl-1-Oxaspiro (4,5) Deca-6,9-Diene-2,8-Dione	C17H24O3	276.376	14.580
20	Methyl Hexadec-9-Enoate	C17H32O2	268.441	14.675
21	Methyl palmitate	C17H34O2	270.457	14.752
22	3-Ethyl-3-pentanol	C14H22O3	238.327	15.108
23	Hexadecanoic Acid	C16H32O2	256.43	15.304

24	Methyl linolelaidate	C19H34O2	294.479	17.055
25	Methyl linolenate	C19H32O2	292.463	17.141
26	Phytol	C20H40O	296.539	17.306
27	Eicosane	C20H42	282.556	17.467
28	Methyl Stearate	C19H38O2	298.511	17.517
29	Linolenic acid	C18H30O2	278.436	17.804
30	Octadecanoic Acid	C18H36O2	284.484	18.084
31	3-(2-Oxocyclohexyl) Propionaldehyde	C9H14O2	154.209	20.351
32	Palmitic acid	C19H38O4	330.509	23.273
33	Glyceryl monostearate	C21H42O4	358.563	27.705
34	Squalene	C30H50	410.73	30.582

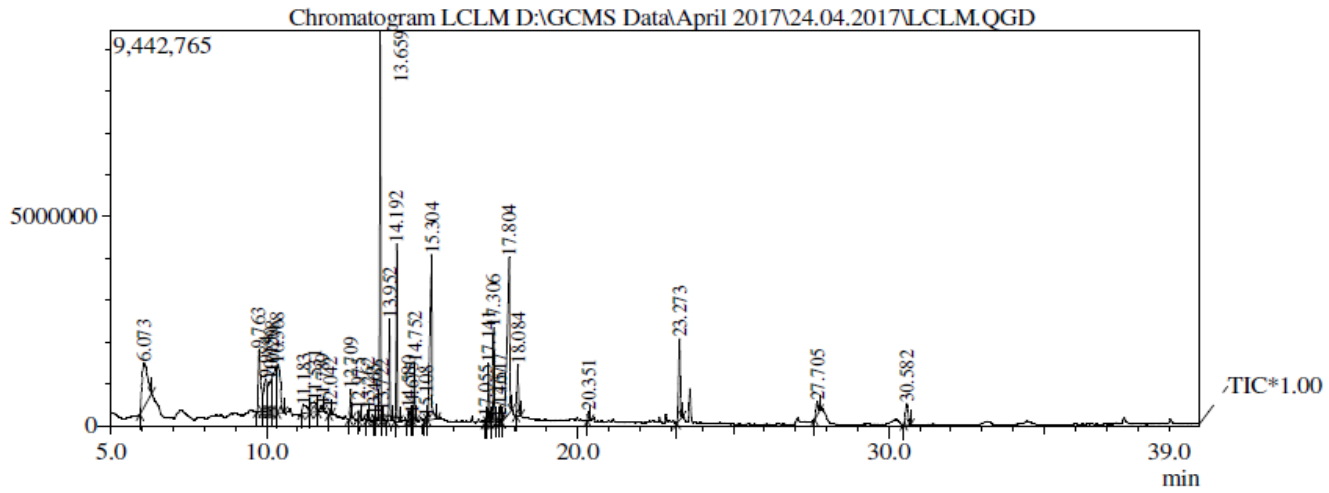

Figure 1 GC-MS - *L. aegyptiaca* - Methanolic Extract of Leaf

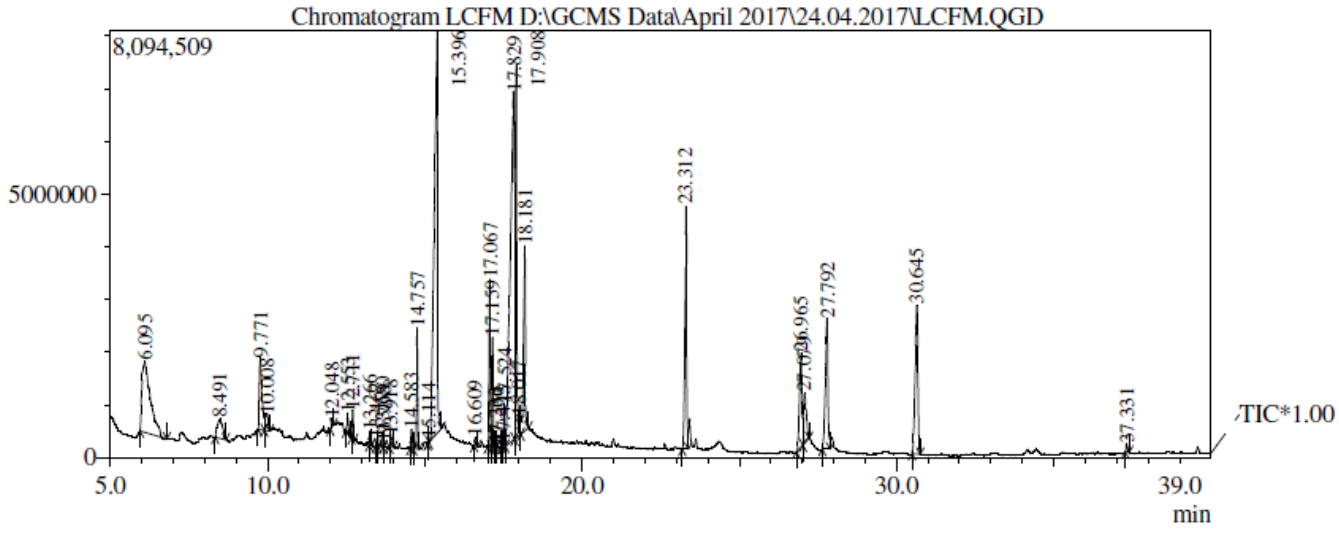

Figure 2 GC-MS - *L. aegyptiaca* - Methanolic Extract of Fruit

Table 2 GCMS - *L. aegyptiaca* - Methanolic Extract of Fruit

S. No.	Name	Molecular Formula	Molecular Weight	Retention Time
1	5-Hydroxymethylfurfural	C6H6O4	142.11	6.095
2	4-Chloroanisole	C8H9ClO	156.609	8.491
3	Phenol, 2,4-bis-(1,1-dimethylethyl), TMS	C14H22O	206.329	9.771
4	Eicosane	C20H42	282.556	12.048
5	Gamma. -Hydroxyisoeugenol	C10H12O3	180.203	12.553
6	Myristic Acid	C14H28O2	228.376	12.711
7	3--Heptadecanol	C17H36O	256.474	13.266
8	Isopropyl Myristate	C17H34O2	270.457	13.467
9	Neophytadiene	C20H38	278.524	13.650
10	Hexahydrofarnesyl Acetone	C18H30O	262.437	13.708
11	Pentadecanoic Acid	C15H30O2	242.403	13.918
12	7,9-Di-Tert-Butyl-1-Oxaspiro (4,5) Deca-6,9-Diene-2,8-Dione	C17H24O3	276.376	14.583
13	Hexadecanoic Acid, Methyl Ester	C17H34O2	270.457	14.757
14	3-Pentanol, 3-Ethyl	C6H14O	102.177	15.114
15	Hexadecanoic Acid	C16H32O2	256.43	15.396
16	Heptadecanoic Acid	C17H34O2	270.457	16.609
17	Methyl 10-Trans,12-Cis-Octadecadienoate	C19H34O2	294.479	17.067
18	9-Octadecenoic Acid (Z)-, Methyl Ester	C19H36O2	296.495	17.159
19	Methyl Dihydromalvalate	C19H36O2	296.495	17.233
20	Phytol	C20H40O	296.539	17.300
21	Phytane	C20H42	282.556	17.475
22	Methyl Stearate	C19H38O2	298.511	17.524
23	Linolelaidic Acid, Methyl Ester	C19H34O2	294.479	17.829
24	Octadec-9-Enoic Acid	C18H34O2	282.468	17.908
25	Cyclobuta [1,2:3,4] Dicyclooctene, Hexadecahydro	C16H28	220.4	18.017
26	Octadecanoic Acid	C18H36O2	284.484	18.181
27	Palmitic Acid Beta-Monoglyceride	C19H38O4	330.509	23.312
28	Beta-Monolinolein	C21H38O4	354.531	26.965
29	Oleoyl Chloride	C18H33ClO	300.911	27.079
30	Alpha-Monostearin	C21H42O4	358.563	27.792
31	Squalene	C30H50	410.73	30.645
32	Gamma-Tocopherol	C28H48O2	416.69	37.331

Table 3 and Table 4 display the outcomes of FT-IR functional groups. The FT-IR spectrum has confirmed the presence of various functional groups such as aliphatic amines, carboxylic acids, aromatics, alkyl halides, primary and secondary amines, amides, alkanes, and alkenes. Figure 3 and Figure 4 depict the FT-IR spectrum profile of leaf and fruit extracts. The results of the antimicrobial activity of leaf and fruit extracts of *L. aegyptiaca* in Acetone, Ethanol, Methanol, and Aqueous forms against ten different bacterial and three fungal strains are presented in Table 5 and Table 6, and illustrated in Figure (5, 6, 7, and 8). The study evaluated the activity of plant organs at various maturation stages. Acetone, ethanol, methanol, and aqueous extracts were tested for activity against gram-positive and gram-

negative bacteria such as *Klebsiella pneumoniae, Enterobacter aerogenes, Proteus vulgaris, Serratia marcescens, Escherichia coli,* and *Proteus mirabilis*. All extracts showed activity against all strains.

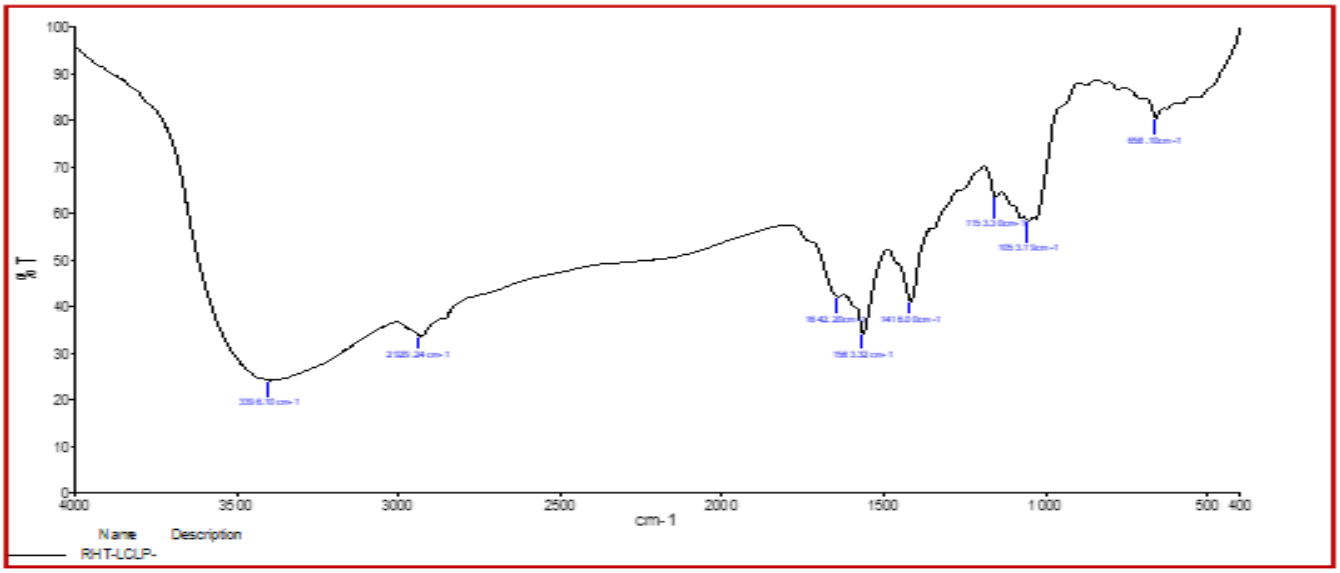

Figure 3 FT-IR- L. aegyptiaca - Methanolic Extract of Leaf

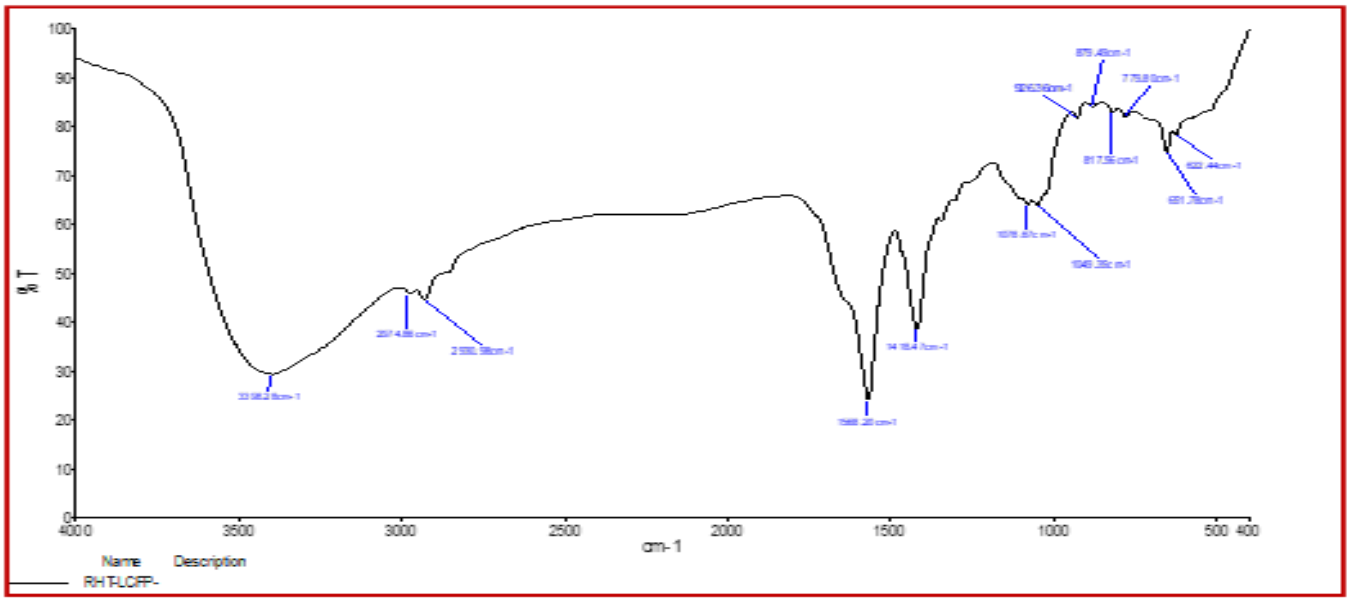

Figure 4 FT-IR- L. aegyptiaca - Methanolic Extract of Fruit

Table 3 FT-IR Spectrum of Leaf of *L. aegyptiaca*

S. No.	Name of the Compound	Group	Stretching Frequency (cm-1)	Molecular Formula
1	Alcohols, Phenols	O-H stretch, H-bonded	3396.10	C2H6O
2	Alkanes	C-H stretch	2929.24	C5H14
3	Alkenes	-C=C- stretch	1642.20	C10H18
4	Aromatics	C-C stretch (in-ring)	1563.32	C14H29N3O4
5	Alkyl Halides	C-H wag (-CH2X)	1153.30	C4H9Cl
6	Aliphatic Amines	C-N stretch	1053.19	C4H11N

Table 4 FT-IR Spectrum of Fruit of *L. aegyptiaca*

S. No.	Name of the Compound	Group	Stretching Frequency (cm-1)	Molecular Formula
1	Primary, Secondary Amines, Amides	N-H stretch	3398.28	C13H29N
2	Alkanes	C-H stretch	2974.86	C5H14
3	Alkenes	=C-H stretch	2930.98	C10H18
4	Aromatics	C-C stretch (in-ring)	1418.47	C14H29N3O4
5	Aliphatic Amines	C-N stretch	1078.67	C4H11N
6	Aliphatic Amines	C-N stretch	1049.35	C4H11N
7	Carboxylic Acids	O-H bend	926.36	C2H4O2
8	Aromatics	C-H “loop”	879.49	C14H29N3O4
9	Alkyl Halides	C-Cl stretch	817.56	C4H9Cl
10	Alkyl Halides	C-Cl stretch	779.80	C22H40N+
11	Alkynes	-C≡C-H: C-H bend	651.78	C2H2
12	Alkyl Halides	C-Br stretch	622.44	C22H40N+

The highest activity of the leaf extract was obtained from the ethanol extract against *Proteus vulgaris* (19.33±1.15), acetone extract against *Proteus vulgaris* (18±1), methanol extract against *Proteus mirabilis* (17.33±1.15), *Serratia marcescens* (15.33±0.57) and the lowest activity was obtained from the acetone extract against *Escherichia coli* (10.33±0.57). The highest activity of the fruit extract was obtained from the methanol extract against *Klebsiella pneumonia* (13.66±1.52), methanol extract against *Proteus vulgaris* (12.33±1.15) and *Serratia marcescens* (12.66±1.52), and the lowest activity was obtained from the ethanol extracts against Escherichia coli (9±1) and aqueous extract against *Escherichia coli* (7.33±0.57).

Table 5 Antimicrobial Assay of Leaf in *L. aegyptiaca*

	Bacteria	Acetone	Ethanol	Methanol	Aqueous	Antibiotic
1	*Enterobacter aerogenes*	-	-	-	-	13.66±1.52
2	*Escherichia coli*	10.33±0.57	12.33±0.57	-	-	21±1
3	*Klebsiella pneumoniae*	-	-	-	-	24.66±1.52
4	*Proteus mirabilis*	15.33±1.15	-	17.33±1.15	-	21.33±0.57
5	*Proteus vulgaris*	18±1	19.33±1.15	-	-	24±1
6	*Serratia marcescens*	15.33±0.57	17±1.73	-	-	20.66±1.52
	Fungi					
1	*Aspergillus niger*	-	-	0.76±0.05	-	-
2	*Penicillium vermiculatum*	-	-	1.11±0.09	-	0.6±0
3	*Mucor indicus*	-	-	1.53±0.05	-	0.71±0.17

Table 6 Antimicrobial Assay of Fruit in *L.aegyptiaca*

	Bacteria	Acetone	Ethanol	Methanol	Aqueous	Antibiotic
1	*Enterobacter aerogenes*	-	-	-	-	14.33±1.15
2	*Escherichia coli*	10.66±1.15	9±1	12.66±1.52	7.33±0.57	17±1.73
3	*Klebsiella pneumoniae*	10.66±1.15	11.66±1.52	13.66±1.52	-	24±1.73
4	*Proteus mirabilis*	7.33±0.57	-	11.66±0.57	-	16.66±0.57
5	*Proteus vulgaris*	-	12.33±0.57	12.33±1.15	-	20.66±0.57
6	*Serratia marcescens*	10.33±0.57	11.33±0.57	12.66±1.52	-	21±1.73
	Fungi					
1	*Aspergillus niger*	-	-	1.35±0.02	-	-
2	*Penicillium vermiculatum*	-	-	1.3±0.06	-	0.58±0.04
3	*Mucor indicus*	-	-	1.46±0.05	-	0.51±0.04

Different methanol extracts were selected and tested for their activity against three fungal strains, namely Aspergillus niger, Penicillium vermiculatum, and Mucor indicus. Results showed that methanol extracts exhibited activity against all three strains. The leaf extract displayed the highest activity against Mucor indicus (1.53±0.05), and the lowest activity against Penicillium vermiculatum (1.11±0.09) and Aspergillus niger (0.76±0.05). On the other hand, the fruit extract showed the highest activity against Mucor indicus (1.46±0.05) and the lowest activity against Aspergillus niger (1.35±0.02) and Penicillium vermiculatum (1.3±0.06).

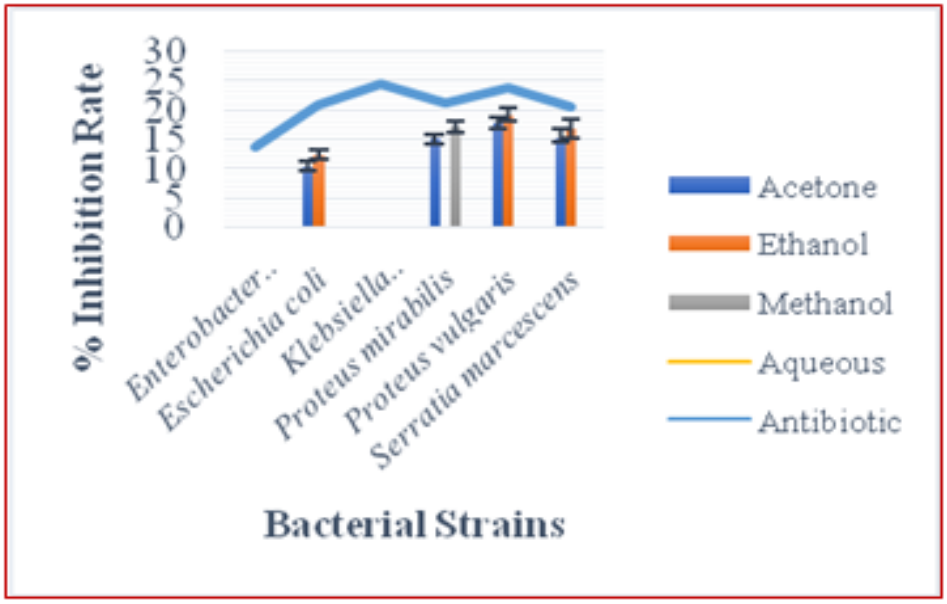

Figure 5 Antibacterial Assay of Leaf in *L. aegyptiaca*

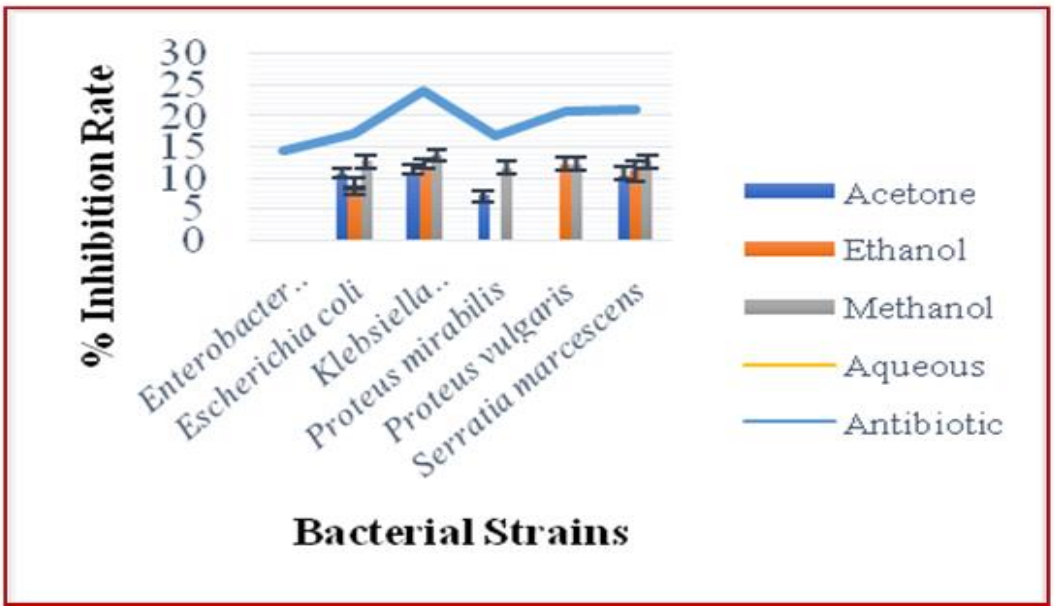

Figure 6 Antibacterial Assay of Fruit in *L. aegyptiaca*

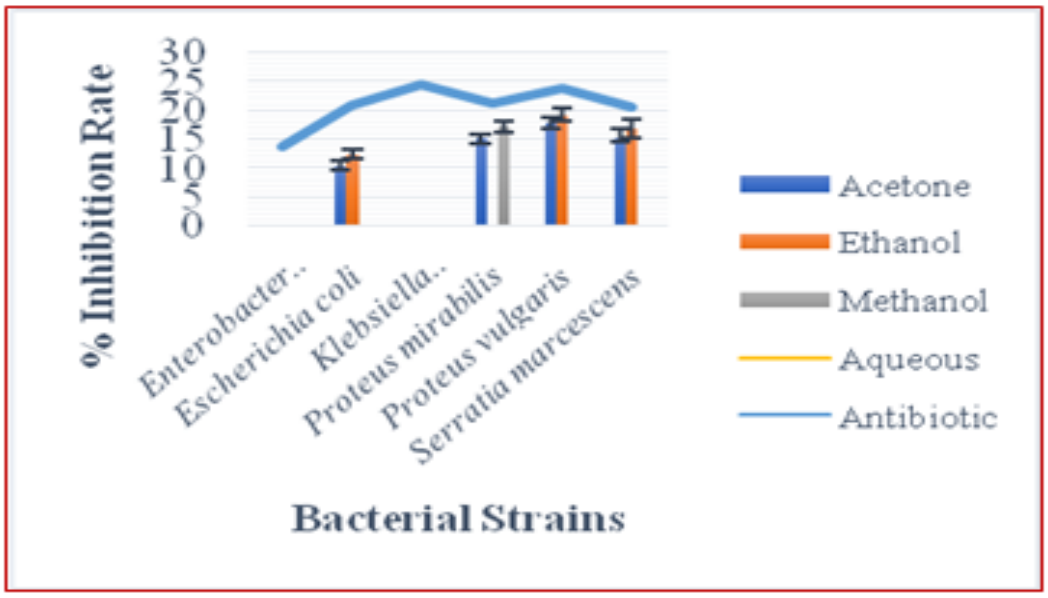

Figure 7 Antifungal Assay of Leaf in *L. aegyptiaca*

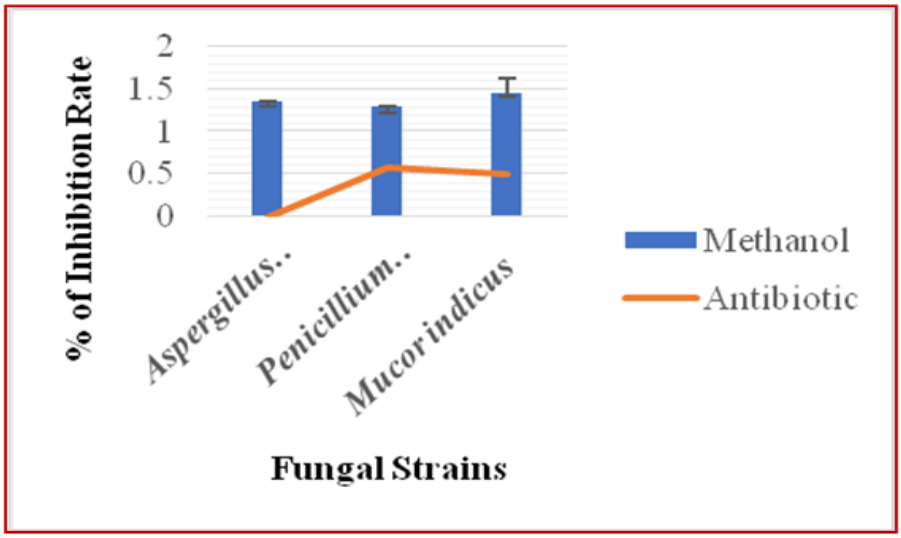

Figure 8 Antifungal Assay of Fruit in *L. aegyptiaca*

4. DISCUSSION

According to a study by Arulraj et al., (2016), many plants belonging to the Cucurbitaceae family possess antimicrobial activity against both gram-positive and gram-negative bacteria. The study found that the crude extracts of these plants showed a wide range of bactericidal properties. This suggests that these plants may be potential sources of antibiotics with a broad spectrum of properties. The results of this investigation confirm the traditional belief that herbs can be used to treat infectious diseases.

5. CONCLUSION

Through phytochemical screening and in vitro antimicrobial studies of *L. aegyptiaca* using GCMS and FT-IR, several compounds [functional groups] including Alcohol, Alkane, Alkane and Acid, Alkene, Alkyl Halide, Nitro, Amine, Ether and Ester were identified. The study revealed that the Acetone extract had significant antibacterial activity in a concentration-dependent manner. The investigation detected the presence of more than five phytochemical substances. Therefore, plant extracts could be used to treat illnesses caused by strains of the tested antibacterial and antifungal organisms.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection & experimentation.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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